Advances in MRS of Diabetes and Obesity

Claudia Neumann-Haefelin
Sanofi-Aventis Deutschland GmbH, Frankfurt, Germany

Insulin Resistance, Diabetes and Obesity

The pathophysiology of type-2 diabetes implies defects in tissue sensitivity to insulin and in an appropriate insulin secretion. Normally, insulin binds to insulin receptors on target organ cells, resulting in a series of cellular events promoting intracellular glucose transport and metabolism. Insulin resistance is defined by the inability of peripheral target tissue to respond properly to normal concentrations of circulating insulin. This is compensated by the pancreas secreting an increased amount of insulin in order to maintain euglycemia. After this first period of compensated insulin resistance, impaired glucose tolerance develops despite elevated insulin concentrations, and insulin resistance amplifies. Finally, the increased insulin secretion results in a progressive pancreatic β-cell dysfunction leading to decreased insulin secretion. Overt clinical diabetes emerges when these two factors, insulin resistance and impaired β-cell function, occur simultaneously (1). Although not all individuals who are insulin resistant will progress to clinical diabetes, the proportion of patients developing diabetes is high.

The molecular mechanisms causing insulin resistance are still not unequivocally elucidated, but there is increasing evidence that glucose and fatty acid metabolism are closely linked and that disturbances of this relationship are associated with insulin resistance (2). One feature of this link is the ectopic accumulation of lipids in non-adipocyte tissue such as muscle and liver, a result of increased free fatty acid lipolysis and diminished esterification and re-esterification of fatty acids in adipose tissue in insulin resistant subjects. Another feature is that defects in insulin-stimulated muscle glycogen synthesis are chiefly responsible for insulin resistance. Hence, by observing metabolic pathways and alterations in carbohydrate and lipid metabolism one can improve our understanding of the molecular mechanisms and the development of metabolic disorders like insulin resistance, obesity and diabetes.

In-vivo NMR Spectroscopy in diabetic and obesity research

In-vivo NMR spectroscopy allows continuous, noninvasive monitoring of tissue concentrations of metabolites and metabolic fluxes in humans and animals. From the broad range of magnetic nuclei, most studies to date have utilized 1H, 31P and 13C to determine muscle and liver glucose and lipid metabolism (3-5).

> _1H-spectroscopy:_ 1H-protons have a natural abundance close to 100% and overall offer the highest sensitivity for NMR observations. In most studies in diabetic and obesity research, fat as one of the strongest signals in the 1H spectrum is analyzed: Liver and muscle lipid signal intensities are related to insulin sensitivity and diabetes (6,7). Whole body fat content is of importance in obesity research (8,9).

31P-spectroscopy: Due to the high natural abundance of 31P (100%), this method has a good sensitivity. 31P-spectroscopy allows evaluation of the bioenergetics of skeletal and cardiac muscle, detecting adenosine triphosphate (ATP), phosphocreatine (PCr) and inorganic phosphate (Pi). Measuring glucose-6 phosphate (G6P), the glucose transport/ phosphorylation activity can be evaluated with detection of significant changes in insulin resistant subjects. (10-14). Furthermore, the abnormal cardiac and skeletal muscle energy metabolism in subjects with type-2 diabetes can be assessed detecting ATP and PCr signals using 31P MRS (15-17).

13C-spectroscopy: In contrast to 1H and 31P, 13C has a natural abundance of only ~1% and therefore a low sensitivity. Nevertheless, it can be used to detect metabolites that exist in high concentrations. For example, 13C spectroscopy enables in vivo assessments of muscle and liver glycogen concentrations (18-21) as well as the observation of triglyceride metabolism (22). Furthermore, 13C-enriched isotopes can be used to enhance the sensitivity in tracer experiments.

In the following, two examples of MRS in diabetes and obesity research will be presented – the observation of intramyocellular lipids using 1H-spectroscopy and the detection of glycogen using 13C-spectroscopy.

Intramyocellular lipids and insulin resistance

The existence of lipid stores in skeletal muscle was first described in 1967 by Denton and Randle in rat muscle (23) followed by Dagenais et al. in 1976 in human forearm muscle (24). A possible relationship between skeletal triglycerides and insulin sensitivity was first proposed by Falholt et al. (25) in 1985 who found increased triglyceride content in skeletal muscle in normoglycemic, hyperinsulinemic dogs. In 1991 Storlien et al. (26) observed that after oversupply of dietary fat the triglyceride stores in rat hind limb muscle increase. This was associated with reduced insulin stimulated glucose uptake.

Currently, the ectopic deposition of fat in non-adipose tissue is considered to be an important aspect in the development of insulin resistance (2,27,28). Muscle triglycerides themselves do not seem to directly interfere with insulin action in the myocytes, but rather serve as a surrogate marker for some other fatty-acid derived metabolite, which acts to impair insulin signalling.

In muscle tissue lipids are stored either as interstitial adipocyte triglycerides (termed extramyocellular lipids / EMCL) or as intramyocellular lipids (IMCL) accumulating as droplets in the cytoplasm of muscle cells. While EMCL is metabolically relatively inert, IMCL stores can be build up, mobilized and utilized within several hours (29-31). Recent data indicate a strong correlation between accumulation of IMCL and insulin sensitivity in humans, not only in diabetics, but also in glucose tolerant and intolerant subjects with or without obesity (32-34).

Classically the determination of muscle triglycerides was only possible by invasive techniques: skeletal muscle biopsy followed by the determination of triglycerides through biochemical extraction (35), histologically with electron microscopy (36) or by

histochemical staining (37). However, using these methods the differentiation of IMCL and EMCL is critical and a large coefficient of variations remains – microscopic methods are semi-quantitative and the investigated volume is only randomly selected; biochemical determination is rather vague due to remaining portions of EMCL in the sample.

Recently, it was shown by Schick et al. (38) and Boesch et al. (39) that it is possible to differentiate between IMCL and EMCL using 1H-spectroscopy. The signals from IMCL and EMCL are separated by a frequency shift of 0.2 ppm. This frequency shift was explained with magnetic susceptibility differences between these two compartments and the anisotropic spatial arrangement of muscle fibers. The chemical shift of the EMCL resonance was shown to be orientation-dependent (39), which is most likely due to the arrangement of adipocytes along muscle fibers. IMCL, on the other hand, is stored in the cytoplasm of muscle cells within spherical droplets (40) resulting in no spatial dependence of their chemical shift on the main magnetic field direction (39). The orientation-dependency of EMCL is essential for the separation of EMCL and IMCL by MRS. Best separation between the two signals is achieved when the investigated muscle lies roughly parallel to the main magnetic field B_0 (39).

The MRS investigation of IMCL most frequently was done using single voxel spectroscopy – in humans (6,7,38,39) as well as in animals (41-43). Voxels were typically located in M. soleus, M. tibialis anterior or M. gastrocnemius avoiding vascular structures and gross adipose tissue deposits. For quantification typically the lipid peaks were scaled to the unsuppressed water peak (at 4.7 ppm) and/or the total creatine peak (at approximately 3.0 ppm) (44,45).

Furthermore, there exist a few papers using MR spectroscopic imaging (MRSI) methods (46-50). However, the relatively small number of studies performed using MRSI is probably due to the strong lipid signal from surrounding subcutaneous fat and bone marrow, which leads to contaminations of the spectra of interest due to signal bleeding at the low MRSI resolution.
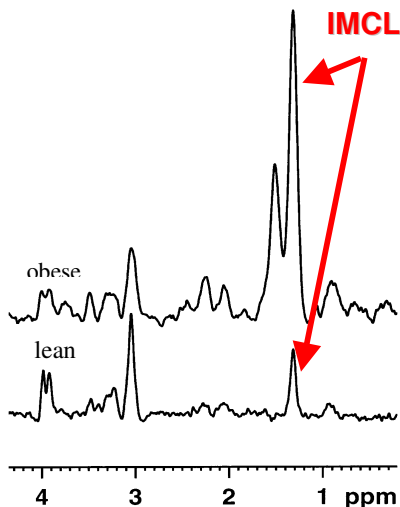

Figure 1: 1H spectra of lean and obese Zucker Diabetic Fatty rats demonstrating the significantly increased IMCL level in insulin resistant animals.

Volume selective ^{1}H MRS/MRSI is currently the only methodology that allows non-invasive monitoring of IMCL levels in vivo. The main reason for the recently increased interest in the measurement of IMCL levels stems from the good correlation between the IMCL concentration and insulin resistance. For example, Krssak et al. (6) observed an inverse relationship between IMCL in the gastrocnemius muscle and insulin-stimulated glucose uptake in a clamp study of 23 normal subjects. A similar relationship was reported by Jacob et al. (7) in insulin resistant, lean, non-diabetic offspring of patients with type-2 diabetes. This correlation between IMCL and insulin resistance in humans could nicely be reproduced in animals (42,43,51). In obese, insulin resistant Zucker Diabetic Fatty rats a significantly higher IMCL level was found than in their lean littermates (see fig. 1). Furthermore, in these publications the effect of treatment with the insulin sensitizer rosiglitazone and its effect on the

IMCL levels were reviewed. Comparing different muscles with the two indices for peripheral insulin sensitivity determined in a glucose clamp study (gold standard to detect insulin resistance), glucose infusion rate (GIR) and glucose disposal (GD), the best correlation between IMCL and insulin sensitivity was found using the M. tibialis anterior and GD (51).

However, up to now it is still unclear which factors influence IMCL formation; its baseline level and / or its turnover. In animal studies it was shown that the main physiological factor influencing IMCL levels is the age of the animals (52). In young rats the IMCL level was much higher than in older ones. This decline in old animals was without recovery to higher IMCL levels in male rats, whereas in female animals IMCL increased again slightly on reaching an age of about 14 weeks. This suggests that sexual maturity (beginning in rats at that age) also influences the IMCL level. Another factor is probably the oxidative capacity of the respective muscle type. Red slow-twitch muscles such as the M. soleus show a higher IMCL content than an intermediate muscle such as the mixed M. tibialis anterior (52). A well known paradox is that endurance trained humans have increased IMCL levels compared to normal subjects despite higher insulin sensitivity (53,54). Only in untrained individuals high IMCL predicts low insulin sensitivity. In trained athletes, this relationship was found to be inverse.

Summarizing, the association between IMCL and insulin resistance is now well established. Under well-defined conditions IMCL can be used as a biomarker to detect insulin sensitivity non-invasively. However, there are a lot of additional factors influencing the IMCL concentration in individuals such as age, gender, physical activity, etc, and therefore, the interpretation of IMCL data requires controlling for these parameters.

Glycogen metabolism

Prior to the introduction of in-vivo NMR spectroscopy, studies in humans to estimate glucose metabolism were limited to either invasive or indirect approaches. Liver or muscle biopsies allow direct quantification of glycogen concentration but are invasive and cannot easily be repeated within one experiment (55). 13C-MRS has made non-invasive and repetitive measurements of hepatic glycogen concentrations in real-time mode possible, permitting the calculation of rates of net hepatic glycogen synthesis and glycogenolysis in vivo. Furthermore, using 13C-labeled glucose experiments can be used to assess rates of hepatic glycogen turnover.

Since the resonance of 13C in the Cl position of glycogen is clearly resolved at 100.5 ppm and all 13C signals from glycogen are detected by 13C-NMR spectroscopy (56), the peak integral of Cl-glycogen (see fig. 2) corresponds to a defined glycogen concentration. Measurements of tissue glycogen content by 13C-NMR spectroscopy have been validated by comparison with muscle and liver biopsies in rabbits (57,58) and muscle biopsies in humans (59). There was an excellent correlation over a broad concentration range of glycogen. Moreover, NMR was found to be more precise than the biopsy measurements.

One of the pioneer experiments that used in-vivo 13C-MRS to evaluate carbohydrate metabolism in skeletal muscle measured directly the rate of human muscle glycogen formation from an isotopically labeled glucose infusion (60). The authors showed that under conditions of imposed hyperglycemia and hyperinsulinemia a majority of the infused

glucose was converted to muscle glycogen in normal men. This directly shows that muscle is the major site of glucose disposal under these conditions, additionally provides quantitation of the glucose flux to muscle glycogen.
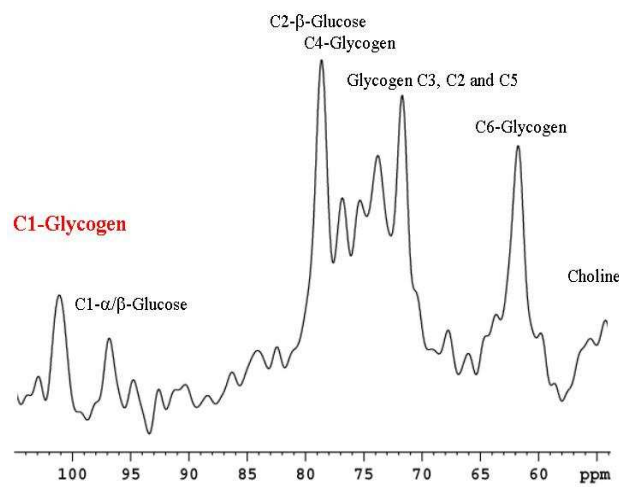

Figure 2: Portion of a proton decoupled 13C natural abundance spectrum of a pig liver ex-vivo at a 4.7T animal scanner.

Shulman et al. (21) and Carey et al. (61) compared changes in muscle glycogen concentration during hyperinsulinemic-euglycemic clamp study in normal controls and subjects with type-2 diabetes. They also conclude that muscle glucose uptake accounts almost completely for the whole body glucose disposal during hyperglycemia, and they designate impaired muscle glycogen deposition as the characteristic defect of glucose disposal for patients with type-2 diabetes.

13C spectroscopy was also successfully applied to the liver. Reports from the group of Shulman (62) have determined hepatic glycogen content to quantify net hepatic glycogenolysis and gluconeogenesis rates after a mixed meal. They found that, even early in the phase of the postabsorptive period when liver glycogen stores are maximal, gluconeogenesis contributes approximately 50% to hepatic glucose production.

These and other studies show that 13C-NMR spectroscopy enables to follow glucose / glycogen metabolism absolutely and non-invasively, and allows to get into different pathways leading to glycogen accumulation in liver and muscle as well as glucose fluxes under normal conditions and in insulin resistant or diabetic patients.

Other techniques applicable for the investigation of glycogen metabolism have strong limitations. For example, the tracer's label can be transferred to other molecules and therefore reenter the respective metabolic pathway. Biopsies are limited to only a few time points, and stress hormone release might alter the concentration of the detected metabolites. A time lag between excision and freeze clamping of tissue may result in overestimation of different metabolic effects. Advantages of 13C spectroscopy include the avoidance of these limitations as well as the possibility to check the region of interest properly by an initial imaging of the volume. On the other hand, the difficulties in establishing 13C NMR are the large chemical shift range and the already discussed low sensitivity. Furthermore, to maximize the signal-to-noise ratio and spectral resolution in 13C NMR spectra, 1H decoupling is generally applied during data acquisition, resulting in a simplified spectral pattern but also in a rise in temperature of the investigated volume of interest. Volume selection in most cases is not necessary, because most in vivo 13C NMR spectroscopy studies of intact organs have been performed using the surface coil as the means to 'localize' the signals.

References:

1. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM. A balanced overview. Diabetes Care 1992;15(3):318-368.
2. McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. Diabetes 2002;51(1):7-18.
3. Jalan R, Taylor-Robinson SD, Hodgson HJ. In vivo hepatic magnetic resonance spectroscopy: clinical or research tool? J Hepatol 1996;25(3):414-424.
4. Cox IJ. Development and applications of in vivo clinical magnetic resonance spectroscopy. Prog Biophys Mol Biol 1996;65(1-2):45-81.
5. Murphy E, Hellerstein M. Is in vivo nuclear magnetic resonance spectroscopy currently a quantitative method for whole-body carbohydrate metabolism? Nutr Rev 2000;58(10):304-314.
6. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. Diabetologia 1999;42(1):113-116.
7. Jacob S, Machann J, Rett K, Brechtel K, Volk A, Renn W, Maerker E, Matthaei S, Schick F, Claussen CD, Haring HU. Association of increased intramyocellular lipid content with insulin resistance in lean nondiabetic offspring of type 2 diabetic subjects. Diabetes 1999;48(5):1113-1119.
8. Stein DT, Babcock EE, Malloy CR, McGarry JD. Use of proton spectroscopy for detection of homozygous fatty ZDF-drt rats before weaning. Int J Obes Relat Metab Disord 1995;19(11):804-810.
9. Kamba M, Meshitsuka S, Iriguchi N, Koda M, Kimura K, Ogawa T. Measurement of relative fat content by proton magnetic resonance spectroscopy using a clinical imager. J Magn Reson Imaging 2000;11(3):330-335.
10. Rothman DL, Shulman RG, Shulman GI. 31P nuclear magnetic resonance measurements of muscle glucose-6-phosphate. Evidence for reduced insulin-dependent muscle glucose transport or phosphorylation activity in non-insulin-dependent diabetes mellitus. J Clin Invest 1992;89(4):1069-1075.
11. Price TB, Perseghin G, Duleba A, Chen W, Chase J, Rothman DL, Shulman RG, Shulman GI. NMR studies of muscle glycogen synthesis in insulin-resistant offspring of parents with non-insulin-dependent diabetes mellitus immediately after glycogen-depleting exercise. Proc Natl Acad Sci U S A 1996;93(11):5329-5334.
12. Shulman RG, Rothman DL, Price TB. Nuclear magnetic resonance studies of muscle and applications to exercise and diabetes. Diabetes 1996;45 Suppl 1:S93-98.
13. Roden M, Shulman GI. Applications of NMR spectroscopy to study muscle glycogen metabolism in man. Annu Rev Med 1999;50:277-290.
14. Shulman GI. Cellular mechanisms of insulin resistance in humans. Am J Cardiol 1999;84(1A):3J-10J.
15. Challiss RA, Vranic M, Radda GK. Bioenergetic changes during contraction and recovery in diabetic rat skeletal muscle. Am J Physiol 1989;256(1 Pt 1):E129-137.
16. Challiss RA, Blackledge MJ, Radda GK. Spatially resolved changes in diabetic rat skeletal muscle metabolism in vivo studied by 31P-n.m.r. spectroscopy. Biochem J 1990;268(1):111-115.
17. Scheuermann-Freestone M, Madsen PL, Manners D, Blamire AM, Buckingham RE, Styles P, Radda GK, Neubauer S, Clarke K. Abnormal cardiac and skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation 2003;107(24):3040-3046.
18. Jue T, Lohman JA, Ordidge RJ, Shulman RG. Natural abundance 13C NMR spectrum of glycogen in humans. Magn Reson Med 1987;5(4):377-379.
19. Avison MJ, Rothman DL, Nadel E, Shulman RG. Detection of human muscle glycogen by natural abundance 13C NMR. Proc Natl Acad Sci U S A 1988;85(5):1634-1636.
20. Beckmann N, Seelig J, Wick H. Analysis of glycogen storage disease by in vivo 13C NMR: comparison of normal volunteers with a patient. Magn Reson Med 1990;16(1):150-160.
21. Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. N Engl J Med 1990;322(4):223-228.
22. Petersen KF, West AB, Reuben A, Rothman DL, Shulman GI. Noninvasive assessment of hepatic triglyceride content in humans with 13C nuclear magnetic resonance spectroscopy. Hepatology 1996;24(1):114-117.
23. Denton RM, Randle PJ. Concentrations of glycerides and phospholipids in rat heart and gastrocnemius muscles. Effects of alloxan-diabetes and perfusion. Biochem J 1967;104(2):416-422.
24. Dagenais GR, Tancredi RG, Zierler KL. Free fatty acid oxidation by forearm muscle at rest, and evidence for an intramuscular lipid pool in the human forearm. J Clin Invest 1976;58(2):421-431.

25.   Falholt K, Cutfield R, Alejandro R, Heding L, Mintz D. The effects of hyperinsulinemia on arterial wall and peripheral muscle metabolism in dogs. Metabolism 1985;34(12):1146-1149.

26.   Storlien LH, Jenkins AB, Chisholm DJ, Pascoe WS, Khouri S, Kraegen EW. Influence of dietary fat composition on development of insulin resistance in rats. Relationship to muscle triglyceride and omega-3 fatty acids in muscle phospholipid. Diabetes 1991;40(2):280-289.

27.   Kelley DE, Mandarino LJ. Fuel selection in human skeletal muscle in insulin resistance: a reexamination. Diabetes 2000;49(5):677-683.

28.   Unger RH, Orci L. Diseases of liporegulation: new perspective on obesity and related disorders. Faseb J 2001;15(2):312-321.

29.   Chalkley SM, Hettiarachchi M, Chisholm DJ, Kraegen EW. Five-hour fatty acid elevation increases muscle lipids and impairs glycogen synthesis in the rat. Metabolism 1998;47(9):1121-1126.

30.   Brechtel K, Dahl DB, Machann J, Bachmann OP, Wenzel I, Maier T, Claussen CD, Haring HU, Jacob S, Schick F. Fast elevation of the intramyocellular lipid content in the presence of circulating free fatty acids and hyperinsulinemia: a dynamic 1H-MRS study. Magn Reson Med 2001;45(2):179-183.

31.   Brechtel K, Niess AM, Machann J, Rett K, Schick F, Claussen CD, Dickhuth HH, Haering HU, Jacob S. Utilisation of intramyocellular lipids (IMCLs) during exercise as assessed by proton magnetic resonance spectroscopy (1H-MRS). Horm Metab Res 2001;33(2):63-66.

32.   Phillips DI, Caddy S, Ilic V, Fielding BA, Frayn KN, Borthwick AC, Taylor R. Intramuscular triglyceride and muscle insulin sensitivity: evidence for a relationship in nondiabetic subjects. Metabolism 1996;45(8):947-950.

33.   Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L. Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H-13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. Diabetes 1999;48(8):1600-1606.

34.   Forouhi NG, Jenkinson G, Thomas EL, Mullick S, Mierisova S, Bhonsle U, McKeigue PM, Bell JD. Relation of triglyceride stores in skeletal muscle cells to central obesity and insulin sensitivity in European and South Asian men. Diabetologia 1999;42(8):932-935.

35.   Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol 1959;37(8):911-917.

36.   Levin K, Daa Schroeder H, Alford FP, Beck-Nielsen H. Morphometric documentation of abnormal intramyocellular fat storage and reduced glycogen in obese patients with Type II diabetes. Diabetologia 2001;44(7):824-833.

37.   He J, Watkins S, Kelley DE. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. Diabetes 2001;50(4):817-823.

38.   Schick F, Eismann B, Jung WI, Bongers H, Bunse M, Lutz O. Comparison of localized proton NMR signals of skeletal muscle and fat tissue in vivo: two lipid compartments in muscle tissue. Magn Reson Med 1993;29(2):158-167.

39.   Boesch C, Slotboom J, Hoppeler H, Kreis R. In vivo determination of intra-myocellular lipids in human muscle by means of localized 1H-MR-spectroscopy. Magn Reson Med 1997;37(4):484-493.

40.   Vock R, Hoppeler H, Claassen H, Wu DX, Billeter R, Weber JM, Taylor CR, Weibel ER. Design of the oxygen and substrate pathways. VI. structural basis of intracellular substrate supply to mitochondria in muscle cells. J Exp Biol 1996;199(Pt 8):1689-1697.

41.   Dobbins RL, Szczepaniak LS, Bentley B, Esser V, Myhill J, McGarry JD. Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. Diabetes 2001;50(1):123-130.

42.   Kuhlmann J, Neumann-Haefelin C, Belz U, Kalisch J, Juretschke HP, Stein M, Kleinschmidt E, Kramer W, Herling AW. Intramyocellular lipid and insulin resistance: a longitudinal in vivo 1H-spectroscopic study in Zucker diabetic fatty rats. Diabetes 2003;52(1):138-144.

43.   Jucker BM, Schaeffer TR, Haimbach RE, Mayer ME, Ohlstein DH, Smith SA, Cobitz AR, Sarkar SK. Reduction of intramyocellular lipid following short-term rosiglitazone treatment in Zucker fatty rats: an in vivo nuclear magnetic resonance study. Metabolism 2003;52(2):218-225.

44.   Kuhlmann J, Neumann-Haefelin C, Belz U, Kalisch J, Juretschke HP, Stein M, Kleinschmidt E, Kramer W, Herling AW. Intramyocellular lipid and insulin resistance: a longitudinal in vivo 1H-spectroscopic study in Zucker diabetic fatty rats. Diabetes 2003;52(1):138-144.

45.   Torriani M, Thomas BJ, Halpern EF, Jensen ME, Rosenthal DI, Palmer WE. Intramyocellular lipid quantification: repeatability with 1H MR spectroscopy. Radiology 2005;236(2):609-614.

46.   Hwang JH, Pan JW, Heydari S, Hetherington HP, Stein DT. Regional differences in intramyocellular lipids in humans observed by in vivo 1H-MR spectroscopic imaging. J Appl Physiol 2001;90(4):1267-1274.

47.   Larson-Meyer DE, Newcomer BR, Hunter GR. Influence of endurance running and recovery diet on

intramyocellular lipid content in women: a 1H NMR study. Am J Physiol Endocrinol Metab 2002;282(1):E95-E106.

48.   Shen W, Mao X, Wang Z, Punyanitya M, Heymsfield SB, Shungu DC. Measurement of intramyocellular lipid levels with 2-D magnetic resonance spectroscopic imaging at 1.5 T. Acta Diabetol 2003;40 Suppl 1:S51-54.

49.   Vermathen P, Kreis R, Boesch C. Distribution of intramyocellular lipids in human calf muscles as determined by MR spectroscopic imaging. Magn Reson Med 2004;51(2):253-262.

50.   Weis J, Courivaud F, Hansen MS, Johansson L, Ribe LR, Ahlstrom H. Lipid content in the musculature of the lower leg: evaluation with high-resolution spectroscopic imaging. Magn Reson Med 2005;54(1):152-158.

51.   Kuhlmann J, Neumann-Haefelin C, Belz U, Kramer W, Juretschke HP, Herling AW. Correlation between insulin resistance and intramyocellular lipid levels in rats. Magn Reson Med 2005;53(6):1275-1282.

52.   Neumann-Haefelin C, Kuhlmann J, Belz U, Kalisch J, Quint M, Gerl M, Juretschke HP, Herling AW. Determinants of intramyocellular lipid concentrations in rat hindleg muscle. Magn Reson Med 2003;50(2):242-248.

53.   DeFronzo RA, Ferrannini E, Sato Y, Felig P, Wahren J. Synergistic interaction between exercise and insulin on peripheral glucose uptake. J Clin Invest 1981;68(6):1468-1474.

54.   Hurley BF, Nemeth PM, Martin WH, 3rd, Hagberg JM, Dalsky GP, Holloszy JO. Muscle triglyceride utilization during exercise: effect of training. J Appl Physiol 1986;60(2):562-567.

55.   Nilsson LH, Furst P, Hultman E. Carbohydrate metabolism of the liver in normal man under varying dietary conditions. Scand J Clin Lab Invest 1973;32(4):331-337.

56.   Shulman GI, Rothman DL, Chung Y, Rossetti L, Petit WA, Jr., Barrett EJ, Shulman RG. 13C NMR studies of glycogen turnover in the perfused rat liver. J Biol Chem 1988;263(11):5027-5029.

57.   Gruetter R, Prolla TA, Shulman RG. 13C NMR visibility of rabbit muscle glycogen in vivo. Magn Reson Med 1991;20(2):327-332.

58.   Gruetter R, Magnusson I, Rothman DL, Avison MJ, Shulman RG, Shulman GI. Validation of 13C NMR measurements of liver glycogen in vivo. Magn Reson Med 1994;31(6):583-588.

59.   Taylor R, Price TB, Rothman DL, Shulman RG, Shulman GI. Validation of 13C NMR measurement of human skeletal muscle glycogen by direct biochemical assay of needle biopsy samples. Magn Reson Med 1992;27(1):13-20.

60.   Jue T, Rothman DL, Shulman GI, Tavitian BA, DeFronzo RA, Shulman RG. Direct observation of glycogen synthesis in human muscle with 13C NMR. Proc Natl Acad Sci U S A 1989;86(12):4489-4491.

61.   Carey PE, Halliday J, Snaar JE, Morris PG, Taylor R. Direct assessment of muscle glycogen storage after mixed meals in normal and type 2 diabetic subjects. Am J Physiol Endocrinol Metab 2003;284(4):E688-694.

62.   Petersen KF, Price T, Cline GW, Rothman DL, Shulman GI. Contribution of net hepatic glycogenolysis to glucose production during the early postprandial period. Am J Physiol 1996;270(1 Pt 1):E186-191.